

Lead and zinc bioavailability to *Eisenia fetida* after phosphorus amendment to repository soils

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Phosphorus form and pH were controlling factors in the effectiveness of phosphorus amendment in decreasing Pb and Zn bioavailability.

Abstract

Four phosphorus forms were investigated as potential soil amendments to decrease the bioavailability of Pb and Zn in two repository soils to the earthworm, *Eisenia fetida*. Treatments were evaluated by examining differences in bioaccumulation factors between amended and non-amended soils. Triple super phosphate at 5000 mg P/kg decreased both Pb and Zn bioavailability in both soils. Rock phosphate at 5000 mg P/kg decreased Zn bioavailability, but not Pb bioavailability in both repository soils. Monocalcium phosphate and tricalcium phosphate at 5000 mg P/kg did not significantly decrease Pb or Zn bioavailability to earthworms in either repository soil. In order to optimize phosphorus amendments, additional phosphorus (up to 15,000 mg P/kg) and lowered pH were used in a series of tests. The combination of lowering the pH below 6.0 and increasing phosphorus concentrations caused complete mortality in all triple super phosphate amended soils and partial mortality in the highest rock phosphate amended soils. Results indicate that triple super phosphate and rock phosphate are viable soil amendments, but care should be taken when optimizing amendment quantity and pH so that adverse environmental effects are not a by-product.

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1. Introduction

Environmental and human health problems worldwide can be attributed to past metal mining and smelting activities. Soil samples obtained from southeast Kansas, southwest Missouri and northeast Oklahoma, collectively known as the tri-state mining area, contain elevated levels of Pb, Zn, and Cd from tailings and

smelting activities. Elevated metal concentrations cause harm to local children and ecosystems. The quantity of Pb in soils has been directly linked to elevated blood Pb concentrations (Lewin et al., 1999) and the abatement of Pb levels to decreases in blood Pb concentrations in 6 to 72-month old children (Lanphear et al., 2003). Residents of the tri-state mining area have exhibited a decline in human health. High levels of Pb have been found in children along with increased prevalence of kidney and heart disease in the tri-state mining area (Nueberger et al., 1990). Exposure to metals can also be hazardous to wildlife and plants. Little or no vegetation exists in old mining regions, causing wind and water erosion to continually increase metal dispersal (Pierzynski et al., 1994).

Soil excavation, solidification, vegetative remediation and phosphorus remediation are used in remediation of

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metal contaminated sites. Locating hazardous waste landfills and high cost are disadvantages to soil excavation. Solidification consists of a cement mix that physically solidifies sediment, while chemically stabilizing and immobilizing contaminants. Although human contamination is reduced, land is rendered unavailable for many flora and fauna. In vegetative remediation, metals may be removed from soils, but they still pose a threat through food web transfer to herbivores. Phosphorus has been shown to immobilize Pb and reduce bioavailability (Ma et al., 1995; Ma and Rao, 1997; Pearson et al., 2000; Hettiarachchi et al., 2001; Cao et al., 2002; Yang et al., 2002; Melamed et al., 2003; Brown et al., 2004). Phosphorus amendments added to contaminated soil can lead to the formation of pyromorphites, an insoluble Pb-phosphate complex. Pyromorphite complexes remain stable in soil as a secondary mineral with phosphate as the limiting formation agent (Maenpaa et al., 2002). High phosphorus treatments reduce the bioavailability of Pb and Zn to earthworms presumably due to the metal-phosphate complexes formed in amended soils (Maenpaa et al., 2002). Ma and colleagues have published many papers on the usefulness of rock phosphate in immobilizing Pb from contaminated soils (Ma et al., 1995; Ma and Rao, 1997; Cao et al., 2002; Cao et al., 2003; Melamed et al., 2003).

Recently, a commercially available biosolid, triple super phosphate (TSP), rock phosphate, and phosphoric acid were utilized alone and in combination in field studies. Addition of 3.2% TSP and 1% phosphoric acid were the most effective treatments for reducing tall fescue grass concentrations of Pb and Zn and in vitro extractable Pb (Brown et al., 2004). Other potential amendments that have been used in an attempt to stabilize Pb in contaminated soils include lime, activated carbon, clay, zeolite, sand, and cement. Lime and cement were significantly effective, while activated carbon, clay, and zeolite were not effective in Pb immobilization as measured by the toxicity characterization leaching procedure (Alpaslan and Yukselen, 2002).

Due to their life history and place in the food web, earthworms are an important soil biota and an effective biomonitoring tool (Neuhauser et al., 1985). Through soil burrowing, earthworms mix organic matter and aerate soils; thereby, increasing their exposure to soil contaminants by direct dermal contact and ingestion. Earthworm bioaccumulation of metals aids trophic

transfer of metals and elevated earthworm metal concentrations have been found in worms inhabiting contaminated soils (Spurgeon and Hopkin, 1996b). Studies have shown decreased earthworm bioaccumulation of Pb and Zn from lab-spiked (Pearson et al., 2000) and field-collected soils amended with TSP or KH_2PO_4 (Maenpaa et al., 2002).

This study was performed in two phases. The first phase evaluated the potential usefulness of a suite of phosphorus forms in decreasing Pb and Zn uptake by the earthworm, *Eisenia fetida*. The second phase took two phosphorus forms that were successful in Phase I and varied the amendment conditions to optimize pH and phosphorus concentrations for limiting Pb and Zn availability.

2. Methods

2.1. Soils and amendments

Test soils from two contaminated repository sites from the Tri-State Mining Region were collected in May 2002. Both the active repository soil (ATR) and time critical repository (TCR) soil were collected from waste landfill sites near Joplin, MO where metal-contaminated soil was stored from nearby urban areas. Soils varied in metal concentrations, but were similar in carbon content (LECO C/N 2000 combustion analyzer, Leco Corporation, St. Joseph, Michigan, USA) and pH (Table 1). Other characteristics that were similar for ATR and TCR soils included %Clay (14 and 18%), cation exchange capacity (13.5 and 13.6 meq/100 g), calcium (2878 and 3035 mg/kg), and potassium (223 and 222 mg/kg). Initial Bray-1 P concentrations for ATR and TCR soils were 136.5 and 467 mg/kg, respectively. Cadmium is a secondary contaminant in ATR and TCR soils (32 and 22 mg/kg). Preliminary studies indicated little affect of P amendments on bioavailability and other authors have found little evidence of Cd immobilization by P addition (Basta et al., 2001), so Cd was not included as an analyte throughout these studies.

Soils were collected in large seed boxes and homogenized by mixing. Soils were transported in 190-L plastic containers from the site and stored at Southern Illinois University (Carbondale, IL, USA) until used for

Table 1

Mean soil total and calcium exchangeable metal concentrations ($n=3$, ± 1 standard deviation), pH and total carbon in active repository (ATR) and time critical repository (TCR) soils

Soil	Total Pb (mg/kg)	Calcium exchangeable Pb (mg/kg)	% Pb exchangeable	Total Zn (mg/kg)	Calcium exchangeable Zn (mg/kg)	% Zn exchangeable	pH	% Total C
ATR	1103 \pm 68	107 \pm 6	9.7%	6592 \pm 503	594 \pm 22	9.0%	7.0	4.74%
TCR	1741 \pm 70	117 \pm 4	6.7%	4655 \pm 1925	399 \pm 21	8.6%	7.0	3.90%

testing. Soils were prepared for bioaccumulation testing after they were sieved through a 2-mm sieve.

Amendments included five phosphorus forms, phosphoric acid (HPLC grade, Fisher Scientific, Fair Lawn, NJ, USA), triple super phosphate (TSP, Voluntary Purchasing Group, Bonham, TX, USA), rock phosphate (RP, IMC Feed Ingredients, Lake Forest, IL, USA), monocalcium phosphate (MCP, BIOFOS, IMC Feed Ingredients, Lake Forest, IL, USA), or tricalcium phosphate (TCP, MULTIFOS, IMC Feed Ingredients, Lake Forest, IL, USA) with pH adjustment with either HPLC grade phosphoric acid or certified ACS Plus hydrochloric acid (Fisher Scientific, Fair Lawn, NJ, USA). After addition of the phosphorus amendment and deionized water to bring moisture content up to ~20%, the soils were mixed by rolling each day for 1 h throughout a 13 d incubation period and stored at room temperature (~22 °C).

2.2. Earthworm cultures

Eisenia fetida were maintained in 380-L plastic cattle troughs in a mixture of peat moss and calcium carbonate (CaCO₃) that was used to buffer the culture substrate to pH 6–7. Earthworm cultures were fed ground oatmeal once a week and maintained at room temperature. Cultures were supplemented from a local supplier (Timberline Fisheries Corp., Marion, IL, USA) as needed. Adult earthworms (clitellum present) were used for all experiments.

2.3. Earthworm bioaccumulation – Phase I

To initiate the tests, approximately 80 ± 2 g of soil (wet weight) and five reproductively mature *E. fetida* were added to each of four replicate 250-mL polypropylene containers for each tested combination and time point. Tests were conducted in a Precision Scientific 818 environmental chamber (Winchester, VA, USA) at 20 °C with continuous light. *E. fetida* are sensitive to light, so the earthworms would burrow in the soil and have a continuous exposure throughout the test. Soil was kept moist by the addition of deionized water on a daily basis.

Time points for these experiments included 0, 12, 24, 48, 96, 144 and 192 h for a total of 28 containers or 140 worms in each soil treatment. At each time point, earthworms from four containers were removed (20 worms maintained as four experimental units), examined for physiological stress, rinsed with deionized water, and allowed to clear soil from their gut for 6 h by returning them to the environmental chamber in clean 250-mL polypropylene containers containing ~2 mL of deionized water. Data collected previously in this lab and repeated prior to this study showed that the main content of the gut is cleared in 6 h (Maenpaa et al., 2002). Gut

clearance is necessary because soil remaining in the gut can cause an overestimate of earthworm metal tissue concentration. According to Hartenstein et al. (1981), 6 h is sufficient time to clear 90% of the gut content for *E. fetida* at 20 °C. Other researchers have reported Zn contributions from gut soil at 1.6% of total *Eisenia andrei* content at 4 h using an isotopic exchange technique (Scott-Fordsmand et al., 2004). Longer gut clearance time may result in decreased metal in earthworm tissue.

A steady-state approach was utilized to calculate bioaccumulation factors (BAFs). BAFs were used as a measure of metal bioavailability in earthworms and were calculated using the equation

$$\text{BAF} = \frac{C_{\text{e}_{\text{ss}}}}{C_{\text{s}}}$$

where, $C_{\text{e}_{\text{ss}}}$ is the metal concentration in the earthworm at steady state (mg metal/kg earthworm) and C_{s} is the total soil metal concentration (mg metal/kg soil). Earthworm steady-state concentrations were calculated from the mean and standard deviation of the four measurements at each of the last two time points ($n=8$, $t=144$ and 192 h) for Phase I or at the 96 h time point ($n=6$) for the Phase II experiments.

2.4. Optimization – Phase II

The test procedure used in the earlier bioaccumulation tests was modified to increase the number of replicate experimental units for each tested combination to six, and decrease the time points to a single 96 h time point, based on Phase I results for all soils and treatment combinations that suggested worm bioaccumulation had reached steady-state. Otherwise, test conditions and amendment methods remained consistent. Three Phase II tests were completed. The first tested whether increased P (15,000 mg/kg) as either TSP and phosphoric acid or RP and phosphoric acid would decrease BAFs in both ATR and TCR soils. Phosphoric acid was used to both increase available phosphorus and decrease pH. The second tested a range of four phosphorus concentrations (as either TSP or RP) from 5000 mg/kg to 12,500 mg/kg at decreased pH in ATR and TCR soil. The final test examined potential pH-caused 96 h acute mortality in ATR soil using 15 total containers (three containers at each of five pHs between 5.7 and 7, light and temperature were consistent with previous bioaccumulation assays).

2.5. Metal analysis

After earthworm gut clearance, worms were rinsed with deionized water to remove any remaining soil from

their exterior, placed in 50-mL polypropylene centrifuge tubes, euthanized by freezing, and dried in a Precision Scientific 45EG oven (Chicago, IL, USA) at 65 °C until dried to a constant weight. Dried earthworms were digested with 10 mL concentrated (16 M) nitric acid for ~2 h in a water bath at 85 °C or until digests were a clear yellow in color. Samples were removed, allowed to cool to room temperature, transferred to 50-mL volumetric flasks and brought to final volume with deionized water.

Soil samples were dried in an oven at 65 °C to a constant weight and digested similarly to the earthworms. After digestion, samples were centrifuged for 15 min in a bench top centrifuge (~1000 g, International Equipment Company Clinical Centrifuge, Needham Heights, MA, USA) and the supernatant diluted to 50 mL with deionized water.

Lead and zinc concentrations in earthworm and soil samples were measured with flame atomic absorption spectroscopy (FAAS, Varian AA Model #220 FS, Palo Alto, CA, USA). All samples were measured within a five point linear calibration prepared daily from 1 mg/mL Acros atomic absorption standards (Fair Lawn, NJ, USA) for each metal and diluted as necessary. Nitric acid digestion blanks were below detection limits (7.1 mg Pb/kg and 11.8 mg Zn/kg) and used at a rate of one per 20 samples.

2.6. Chemical extraction

A chemical extraction method was used to measure potentially available metal concentrations and provide a comparison to earthworm bioavailable metal only during the first Phase II study. Calcium exchangeable metal was used as a surrogate for bioavailable metal (Voegelin et al., 2003). Soil and a 1.0 M CaCl₂ solution were combined in a 10:1 solution to soil ratio (SSR, in mL/g) for 24 h, centrifuged for 5 min in a bench top centrifuge (~1000 g) and the supernatant removed without disturbing the soil pellet for FAAS analysis.

2.7. Statistical analyses

An analysis of variance model was performed to detect amendment differences for the Phase I bioaccumulation tests with PROC GLM of the SAS statistical package (SAS® Institute, Cary, NC, USA). A Dunnett, one-tailed *t* test was used to evaluate significant differences ($\alpha=0.05$) from control. The lethal concentration for 50% of the organisms (LC50) was calculated using PROC PROBIT of the SAS statistical package for the Phase II pH mortality test.

3. Results and discussion

3.1. Earthworm bioaccumulation – Phase I

Phosphorus form and pH significantly influenced the effectiveness of soil amendments in decreasing bioavailable Pb and Zn to *E. fetida*. In the first part of our study, phosphorus form was the main factor controlling whether there were significant differences in BAF from non-amended soils. Earthworm body residues for Pb and Zn in both ATR and TCR soils generally reached steady-state concentration within 48 h (Fig. 1). Therefore, a steady-state approach using the last two time points (144 and 192 h) was used to estimate BAF values for the Phase I studies in a manner similar to that previously reported (Maenpaa et al., 2002). Average background metal concentrations ($n=47$) for the earthworms were 108 ± 32 mg/kg for Zn and below method detection limits of 7.1 mg/kg for Pb.

As expected, in both ATR and TCR soils, TSP significantly decreased earthworm BAF values for both Pb and Zn (Table 2). Rock phosphate significantly decreased BAF values for Zn, but not for Pb. Triple

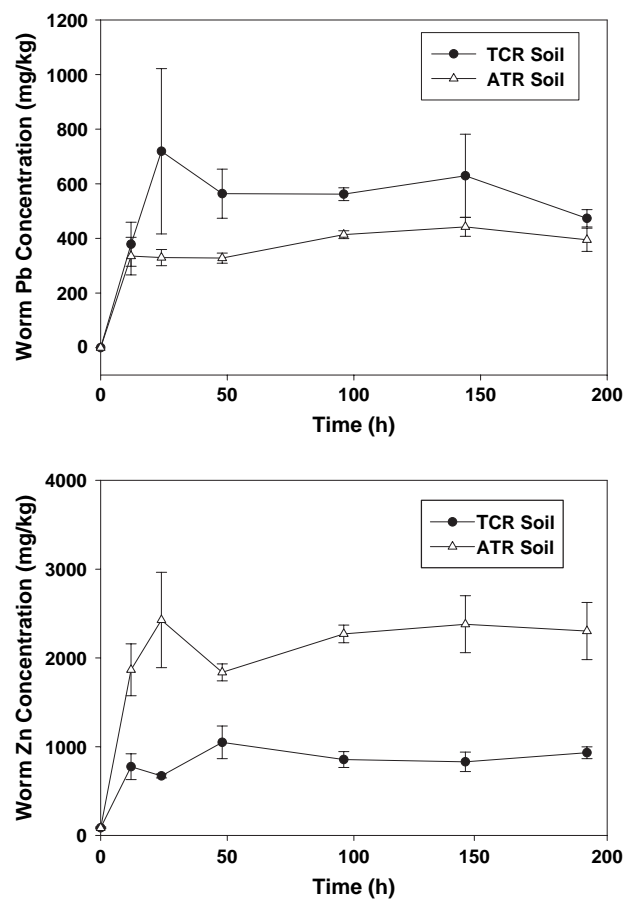


Fig. 1. Mean ($n=4$, ± 1 standard error) concentrations of Pb and Zn in *Eisenia fetida* over time for non-amended active repository (ATR) and time critical repository (TCR) soils.

Table 2

Bioaccumulation factors (BAFs ± 1 standard deviation) for active repository (ATR) and time critical repository (TCR) soils, for each metal and soil treatment ($n=8$ experimental units, $t=144$ and 192 h for each treatment).

	Treatment ^a	ATR BAF	TCR BAF
Pb	None	0.35 \pm 0.05	0.27 \pm 0.03
	TSP	0.06 \pm 0.04*	0.02 \pm 0.01*
	RP	0.51 \pm 0.11	0.32 \pm 0.01
	MCP	0.34 \pm 0.07	0.30 \pm 0.04
	TCP	0.31 \pm 0.08	0.33 \pm 0.05
Zn	None	0.34 \pm 0.06	0.21 \pm 0.03
	TSP	0.09 \pm 0.03*	0.09 \pm 0.04*
	RP	0.10 \pm 0.01*	0.12 \pm 0.01*
	MCP	0.25 \pm 0.04	0.16 \pm 0.03
	TCP	0.31 \pm 0.12	0.16 \pm 0.05

*Significantly different ($\alpha=0.05$) from non-amended soil.

^a Phosphate treatments were normalized to 5000 mg P/kg soil using triple super phosphate (TSP), rock phosphate (RP), monocalcium phosphate (MCP), and tricalcium phosphate (TCP).

super phosphate and RP have been utilized to immobilize Pb in many previous studies (Ma et al., 1997; Hettiarachchi et al., 2001; Maenpaa et al., 2002; Melamed et al., 2003; Brown et al., 2004). Bioaccumulation of Pb and Zn is thought to be decreased due to the formation of Pb and Zn phosphate complexes in phosphorus-amended soils. The formation of Pb and Zn phosphorus complexes such as chloropyromorphite [$\text{Pb}_5(\text{PO}_4)_3\text{Cl}$], decreases the bioavailability of Pb. The range of bioavailability of total soil Pb can range from 90% to less than 10% depending on the mineral form of Pb present (Ruby et al., 1999). Formation of pyromorphite and other metal-phosphorus complexes was demonstrated under laboratory conditions with the addition of phosphorus as TSP or RP, and Pb in various forms, including Pb salts, Pb minerals, and Pb-contaminated soils (Pearson et al., 2000; Hettiarachchi et al., 2001; Ryan et al., 2001; Zhang and Ryan, 2004).

In our RP amended soils, we found it surprising that RP did not significantly alter the Pb BAFs, especially considering the significant literature that had previously shown RP to immobilize Pb by forming pyromorphite like minerals in both laboratory (Ma et al., 1995) and field (Cao et al., 2003) studies. Molar ratios of phosphorus to Pb for our study were 15 and 9.5 for ATR and TCR soils, respectively (Table 3). These

phosphorus: metal ratios were higher than the phosphorus:Pb ratio of four reported by Cao et al. (2003). However, the phosphorus:Zn ratio they used was 7.5 and for both of our soils it was significantly less, with ratios of 0.1 and 0.06 for ATR and TCR soils, respectively. Although the effectiveness and mechanisms of using phosphate to immobilize Zn are not well understood, there are several potential mechanisms currently under study (Cao et al., 2003). Since there was significantly more Zn in our system than Pb (19:1 Zn:Pb molar ratio for ATR and 8.5:1 molar ratio for TCR), Zn probably out competed Pb for the phosphorus amendments at 5000 mg P/kg. This is consistent with a decrease in Zn BAF values for both TSP and RP, but only a decrease in Pb BAF values for TSP, since TSP contains a more available form of phosphorus and tends to slightly acidify soil when used as an amendment. The combination of low phosphorus:Zn ratio and interest in the effects of pH on phosphorus amendments led us to the Phase II set of experiments.

Neither of the calcium phosphate amendments (MCP or TCP) decreased Pb or Zn bioavailability to earthworms, probably because the fraction of Pb or Zn that was calcium exchangeable in both soils was less than 10% of the total metal concentration (Table 1). Calcium competes with Pb and Zn for absorption sites on clay minerals, oxides, and soil organic matter in acidic soils, therefore calcium solutions have been used for modeling cation-exchange in soils (Voegelin et al., 2001). Weak-electrolyte extractions such as those with $\text{Ca}(\text{NO}_3)_2$ -extractable metal have shown promise as a surrogate measure of Zn bioavailability for earthworms in soil (Conder et al., 2001).

3.2. Optimization – Phase II

The first of the Phase II experiments was designed to test a higher phosphorus amendment in soil where the pH had been lowered using phosphoric acid. In field experiments, RP was successfully used as an amendment to immobilize Pb in situ by spreading it after phosphoric acid application (to decrease pH and increase solubility of the RP) (Cao et al., 2003). Therefore, we increased our phosphorus concentration to 15,000 mg/kg and decreased the pH by adding phosphoric acid. This led to several unexpected consequences. The first of these was complete earthworm mortality in all 15,000 mg P/kg treatments in which the phosphorus source was TSP and 60% mortality in the 15,000 mg P/kg treatment with RP as the phosphorus source at decreased pH (Table 4). The second was that even at 5000 mg P/kg, where we had not seen mortality previously at pH 6.4, with pH decreased to 5.9 there was partial mortality with TSP amendment. Conder et al. (2001) had less than 15% mortality in their reference soil amended with $\sim 13,500$ mg P/kg at pH 7. BAF values calculated for treatments that had less than

Table 3

Phosphorus to metal ratios for active repository (ATR) and time critical repository (TCR) soil amended with either 5000 or 15,000 mg P/kg compared to that of Cao et al. (2003)

	Cao et al. (2003)	ATR Soil		TCR Soil	
		5000 mg/kg	15,000 mg/kg	5000 mg/kg	15,000 mg/kg
P:Pb	4.0	15	90	9.5	55
P:Zn	7.5	0.1	4.7	0.06	6.7

Table 4

Bioaccumulation factors (BAFs ± 1 standard deviation), pH, and phosphorus concentration (mg P/kg) for phosphorus (P) and pH modified Time Critical Repository soil for Pb and Zn

	P source ^a	P Conc.	pH	BAF or % mortality ^b
Pb	TSP	5000	6.4	0.22 \pm 0.05
	TSP	5000	5.9	56%
	TSP	15,000	6.5	100%
	TSP	15,000	5.4	100%
	RP	5000	7.1	0.23 \pm 0.05
	RP	5000	6.0	0.24 \pm 0.02
	RP	15,000	7.3	0.23 \pm 0.05
	RP	15,000	5.4	60%
Zn	TSP	5000	6.4	0.13 \pm 0.01
	TSP	5000	5.9	56%
	TSP	15,000	6.5	100%
	TSP	15,000	5.4	100%
	RP	5000	7.1	0.17 \pm 0.03
	RP	5000	6.0	0.16 \pm 0.3
	RP	15,000	7.3	0.15 \pm 0.2
	RP	15,000	5.4	60%

^a Triple super phosphate (TSP) and rock phosphate (RP) were used as the phosphorus source.

^b Mortality reported if greater than 10%.

10% mortality were similar to those in the Phase I experiments (Table 2). In treatments with greater than 10% mortality even though the worms were not dead, they showed signs of poor health, such as no reaction when prodded with forceps or remaining on top of the soil instead of borrowing into the soil matrix, and most importantly did not purge their gut contents during the 6 h clearance time. If BAFs were calculated from unhealthy worms, they would likely be incorrect due to high metal concentrations in the soil remaining in the unpurged earthworm gut.

Elevated phosphorus, decreased pH, or a combination of both potentially caused the mortality. Edwards (1988) reported that *E. fetida* could tolerate a pH range from 4.0 to 7.0, therefore a phosphorus dose response experiment at low pH was designed that included both a soil-only control and a decreased pH (with HCl) control to evaluate the possibilities. All soil samples with pH less than 6 had some mortality (27–100%) or evidence of poor worm health such as those mentioned above. Bioaccumulation factors for Pb and Zn were the same as those previously calculated for TCR soil alone (Table 2). The Pb BAF for 12,500 mg P/kg RP amended TCR soil was lower (0.20 \pm 0.08) than non-amended soil, but not significantly different. All TSP treatments (range of 5000–12,500 mg P/kg) had 100% mortality at pH less than 6.0. Elevated phosphorus concentrations, pH, or a combination of both caused mortality in this set of experiments.

A final pH-only test was performed which resulted in an LC50 for *E. fetida* in ATR soil at pH 6.2. Mortality was potentially due to acute acid stress, which occurs when earthworms lose large amounts of electrolytes due to excess H⁺, absorbed from soil or water by the

integument (Rusek and Marshall, 2000). A second potential cause of mortality was that at lowered pH, bound metal was released, increasing the toxicity due to either Pb or Zn individually or more likely a combination of stress due to both. Total Zn in both ATR and TCR soils was much greater than 620 mg/kg, a reported LC50 value for total Zn in artificial soil amended to 5% organic matter content (Spurgeon and Hopkin, 1996a).

4. Conclusions

Comparison of earthworm BAF values assessed reductions in metal bioavailability and phosphorus immobilization effectiveness. Utilizing the BAF approach, TSP and RP were effective amendments for reducing metal bioavailability to earthworms in contaminated repository soils. Phosphorus form and pH were both important in the utilization of phosphorus-amendments for decreasing bioavailable metal. Significant reductions of Pb and Zn BAFs with TSP and Zn with RP amendments were observed. However, acidification and increasing phosphorus concentrations caused unexpected organism mortality. Acidification of soils to increase the effectiveness of RP amendments will affect terrestrial systems not only by releasing phosphorus and metal ions to increase the formation of pyromorphites, but also releases excess H⁺ in the process (Cao et al., 2002) that can interfere with organism homeostasis (Rusek and Marshall, 2000). Field studies utilizing various phosphorus forms, performed to date, have examined immobilization of Pb via chemical extraction methods, but have not investigated the overall impact on the terrestrial biota, some of which may have adapted to these metal impacted environments (Spurgeon and Hopkin, 2000). Phosphorus amendment has great potential for decreasing bioavailable Pb and Zn, but future research should focus on potential adverse affects due to acidification necessary for formation of pyromorphites from RP and contributions to watershed eutrophication due to phosphorus run-off from amended metal contaminated sites.

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